

Micro CT Assessment of Stem Cell-Mediated Bone Regeneration

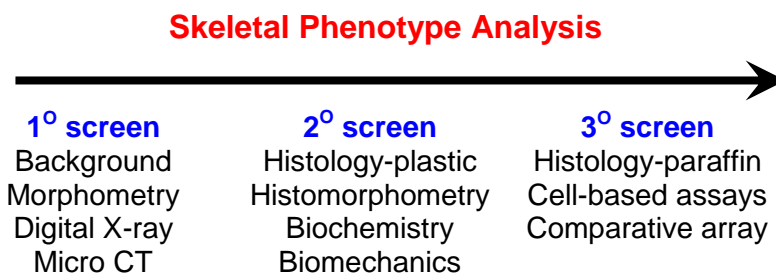
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Background Enormous resources have been expended in the academic and private sectors over the past two decades to generate models of human disease by introducing mutations into the mouse genome. The success of these genetic-based approaches to predict susceptibility or resistance to disease relies heavily on the ability to accurately and reliably characterize the phenotypes associated with a specific disease. From 2003-2008 our laboratory developed phenotyping protocols that correlated micro CT imaging with the histological and molecular analysis of skeletal development (1-11).

Fig 1 Skeletal Phenotyping of Mutant Mice



Over the past two years we have modified these protocols to evaluate bone regeneration in mice previously characterised with osteopenic disorders resembling human osteoporosis. The objective of our ongoing work is to identify novel adjunct therapies for assisted bone regeneration in the presence of poor bone quality using micro CT as a front line screening tool to quantify new bone formation.

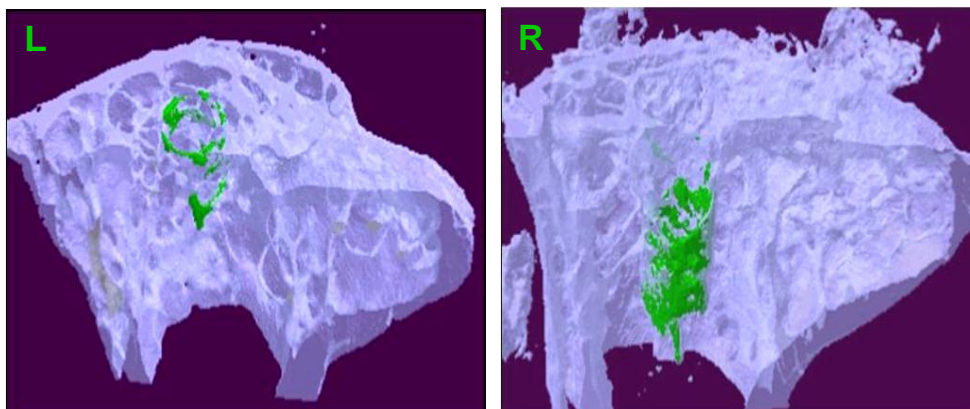
Study I Development of a Model for Implant Fixation. Replacement of joint is the most common major orthopaedic procedure in our ageing population and its long-term success is critically dependent on early fixation of the prosthetic device in host bone (12). This is accomplished by native bone ingrowth in young patients but the decline in regenerative capacity in older patients requires that the implant is fixed with cement, resulting in inflammation, bone cell toxicity and implant loosening. This study was designed to determine if mesenchymal stem cell (MSC) transplantation improves implant osseointegration in a model of age-related bone disease.

Methods Smooth nylon rods measuring 0.4 mm x 10 mm were evenly coated with a 150 nm layer of titanium by physical vapour deposition at the McGill Institute for Advanced Materials. The hind limbs of five month old osteopenic FGFR3^{-/-} recipient mice were irradiated with 13.5 Gy to ablate endogenous MSC. Two days later 10⁵ MSC from age matched FGFR3^{+/+} donor mice were injected via the

pyriformis fossa into the right femoral canal, while the left femur received carrier alone, before inserting the titanium-coated implants. The mice were euthanised after six weeks and the femurs harvested for micro CT and for histological analyses. The Skyscan 1172 currently in use at our institution is equipped with an x-ray source of maximum power 10w and 100 kV. A 10 mega pixel camera, micro-positioning stage and NRecon software enable scanning and 3D reconstruction of specimens measuring approximately 7.0 cm x 3.5 cm at a resolution of up to 8,000 x 8,000 pixels and a detection limit of 0.7 μm isotropic detail. After scanning the femora are processed for embedding in polymethylmethacrylate at low temperature, to preserve enzyme activity, and sectioned at 5 for histochemical and immunochemical staining as described previously (1-4, 6, 10).

Results A peri-implant cylinder measuring 0.6 mm x 2 mm around the titanium coated implant in the proximal metaphysis was identified as the region of interest for micro CT analyses of newly formed bone. Fig 2 shows a significant increase in bone formation was seen in the RIGHT (R) femur that received the MSC transplant compared with the LEFT (L) femur that received carrier alone. The newly formed bone had increased trabecular connectivity and superior structural properties compared with the native bone seen in FGFR3^{-/-} mice. Histological analyses confirmed the increase in bone formation, with a concomitant reduction in fibrous tissue formation, suggesting MSC transplantation represents a potential adjunct therapy to improve implant fixation in the presence of poor quality bone.

Fig 2 Trabecular bone formation around titanium coated implant

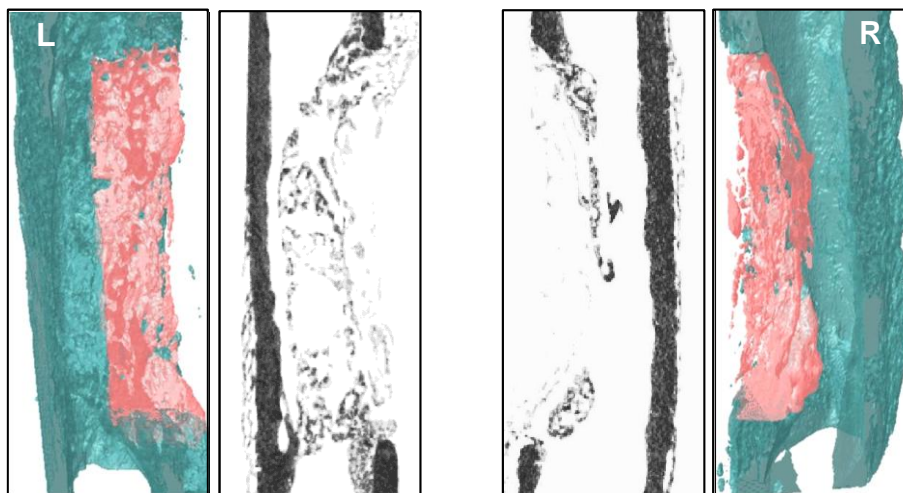


Study II Development of a Model for Fracture Repair. Delayed or failed bone repair leads to mal-union or non-union in up to 20% of patients who have sustained fractures. In older patients it is most commonly associated with an overall decline in the availability of cells and biological agents in the bone micro-environment that are needed for tissue repair (13). Despite advances in the surgical management of fractures, costly and debilitating complications resulting from delayed fracture healing continue to increase with the mean age of the population. Current therapeutic options to enhance bone regeneration, which include bone grafting and protein-based therapy, do not address the significant challenges associated with the reconstruction of large nonunion defects. This study was designed to determine if MSC encased in a dense collagen scaffold could promote healing of a cortical bone defect.

Methods MSC isolated from four month old wild type C3H donor mice were expanded *ex vivo*, seeded into hydrated type I collagen, which was subjected to unconfined compression to generate dense collagen scaffolds. The cell-seeded scaffolds were then cultured for up to 21 days. MSC viability was monitored using the AlamarBlue® metabolic assay and differentiation into osteoblasts using alkaline phosphatase (ALP) and von Kossa staining. Window defects measuring 1mm x 3mm were drilled bilaterally in the femurs of elderly recipient C3H mice with the left femur receiving a dense cell-seeded scaffold and the right femur an acellular scaffold. The quantity and quality of bone regeneration in the defects was assessed after 2 and 4 weeks using micro computed tomography (mCT) and histology as described above.

Results The dense collagen scaffold had superior mechanical properties and supported the survival and differentiation of MSC into osteoblasts up to 21 days in culture. Cells in uncompressed gels and those in compressed gels in non-osteogenic medium, had fewer ALP-positive cells at early time point and less mineral deposited at later times compared with those in compressed gels in osteogenic medium. Fig 3 shows cell-seeded dense collagen scaffold in the LEFT femoral defect resulted in higher BV/TV, Tb.N. and trabecular connectivity compared with the RIGHT defect containing the acellular dense collagen scaffold. The difference is also apparent in 2D (black and white) cuts from the mid-sagittal plane. Histological analyses revealed that the dense collagen scaffold acted as a barrier to bone regeneration in the defect by endogenous MSC but supported osteogenic differentiation and bone formation by transplanted MSC. The work suggests that MSC-seeded dense collagen scaffolds could be used as adjunct therapy to be used with bio-inert allograft bone to promote bone regeneration and repair in large skeletal defects.

Fig 3 MSC-seeded Scaffold for Assisted Bone Repair



Conclusion Our research team at the JTN Wong Laboratories was instrumental in validating the use of computed tomographic imaging as a rapid and viable alternative to traditional histomorphometric analyses as the gold standard for the phenotypic analysis of bone architecture. The first generation Skyscan 1072 is still used for routine analyses on a fee-for-service basis while the Skyscan 1172 is used to develop new protocols for tissue engineering applications. When combined with histological analyses of undecalcified tissues this provides a rapid and comprehensive approach to a wide variety of pre-clinical applications for regeneration and restoration of the skeleton.

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